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Effect of penicillin G-induced epileptic seizures on hemorheological parameters in rats

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Abstract

Normally, cerebral blood flow (CBF) is quantitatively coupled to cerebral metabolic rate like other tissues and maintained basically by altering vascular geometry and appropriate perfusion pressure. However, the rheological properties of the blood are important factors for effective tissue perfusion. Although a lot of studies have reported that hemorheological parameters are affected by a wide range of pathophysiological conditions, to our knowledge no research related to the effects of epileptic seizures on hemorheological parameters has been carried out. Thus, the aim of this study was to explore possible changes in rheological parameters including red blood cell (RBC) deformability, rigidity and aggregation, whole blood and plasma viscosity during epileptic seizures induced by penicillin G in rats. Eighteen female albino rats were divided into three groups that included sham operated controls (Group S), epileptic group (Group E), intraperitoneal penicillin group (Group IPP). Epilepsy was induced by intracortical injections of penicillin G. Hemorheological studies had been carried out 3 h after the induction of epilepsy. Among the studied hemorheological parameters, only RBC deformability was found to be different in the E group compared to S group. Epileptic seizures led to an increase in RBC deformability in the E group. In conclusion, these results suggest that in addition to an increase in CBF, RBC deformability may also improve to better match brain metabolic demands during seizures.

Keywords: Penicillin G; Epileptic seizures; Hemorheology

Introduction

Epilepsy is recognized as the commonest serious neurological disorder in the world. It is characterized by short, recurrent, periodic attacks of motor, sensory, or psychological malfunction (Meldrum and Bruton, 1992). It has been shown that the attacks, called epileptic seizures, afflict about 1% of world's population. Epileptic seizures have many causes, including brain damage at birth; metabolic disturbances (hypoglycemia, hypocalcemia, uremia, hypoxia); infections (encephalitis); toxins (alcohol, tranquilizers, hallucinogens); vascular disturbances (hemorrhage, hypotension) and tumors (Tortora and Grabowski, 2003). However, most epileptic seizures have no demonstrable cause. They are initiated by abnormal, synchronous electrical discharges from millions of neurons in the brain. The discharges stimulate many of the neurons to send nerve impulses over their conduction pathways (Straub et al., 2000). The two hallmarks of seizure generation are hyperexcitability of neurons and hypersynchrony of neural circuits (Stafstrom, 2006). There are numerous seizure types and numerous mechanisms by which the brain generates seizures. Basically, they are classified as to whether their onset is partial, beginning in a small focus on one side of the brain and producing milder symptoms, or generalized seizures involving larger areas on both sides of the brain and loss of conscious (Fisher, 1989).

Effective tissue metabolism and function are dependent on effectual blood supply, and tissues are well-equipped with vascular control mechanisms. Control of blood flow is directly related to the metabolic conditions of the tissue and normally functions to match the metabolic demands of the tissue with the blood flow supply to the tissue (Baskurt and Meiselman, 2003). In accordance with this, focal increases in cerebral blood flow (CBF) have been reported to be associated with the degree of propagation of epileptic activity during partial seizures both in

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humans (Duncan, 1997) and in animals (Goto et al., 1994; de Vasconcelos et al., 2005). Increasing CBF during seizures probably is due to matching neuronal activity and preserving energy supplies to the brain (Busija and Leffler, 1989; Pourcyrous et al., 1992). In addition to increases in CBF, numerous reports are also available concerning cardiovascular manifestations such as blood pressure, ECG changes, heart rate and rhythm in epilepsy (Freeman, 2006).

It has also been suggested that the blood is being affected in status epilepticus (Akbas et al., 2005; Kumar et al., 2005). Although blood flow to the brain is mainly controlled by vascular geometry (i.e., diameter) as well as other tissues, the importance of the rheological properties of blood including whole blood and plasma viscosity, red blood cell (RBC) deformability, and RBC aggregation as determinants of vascular flow resistance have only recently been appreciated (Baskurt and Meiselman, 2003). Since there is no report concerning the effect of experimental epilepsy on hemorheological parameters in rats, we aimed to explore possible effects of epileptic seizures on rheological parameters including plasma viscosity (PV), whole blood viscosity (WBV), RBC deformability, rigidity index (Tk) and aggregation in penicillin-induced partial status epilepticus in rats.

Material and methods

Animals

Sprague–Dawley rats of 16–20 weeks of age (Pamukkale University Experimental Animal Laboratory, Denizli, Turkey) were housed in groups of 4–5 per cage (42×26×15 cm) in a room with controlled temperature (23±2 °C) and relative humidity (60±5%) with lights on from 7:00 to 19:00. Food and water were available ad libitum. Animal handling during all experiments was consistent with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23) and approved by the Pamukkale University Ethics Committee of Animal Care and Usage. A total number of eighteen adult female rats were used in this study. Animals were divided into three groups as follows: 1 — Sham operated group, received saline intracortically (S, $n=6$), 2 — Penicillin group, received saline intracortically and penicillin intraperitoneally (IPP, $n=6$) and 3— Epilepsy group, received penicillin intracortically (E, $n=6$).

Induction of penicillin G epilepsy

For intracortical injections, the rats were anaesthetized with xylazine (10 mg/kg, i.p.) and ketamine (90 mg/kg, i.p.) and the skull was fixed in a stereotaxic device (Stoelting, Wood Dale, IL, USA) after shaving the scalp. The coordinates of the point of intracortical injection (mm) applied were relative to the skull surface, with the upper incisor bar 3.4 mm below the level of the interaural line, according to Paxinos and Watson (1986): posterior to bregma AP=−2 mm; right to the midsagittal line, $L=2$ mm, and dorsoventral, DV=2 mm. The scalp was cut and a hole was drilled at this point on the calvaria. Penicillin G (1000 IU/1 μl distilled water) was intracortically injected by a Hamilton Injector to develop epileptic seizures. The seizures were observed in 5–6 min. Animals were maintained under standard laboratory conditions. 3 h after the induction of epilepsy, heparinized blood was collected from the abdominal aorta of rats under xylazine and ketamine anesthesia and used for the determination of hemorheological parameters including RBC deformability and aggregation, whole blood and plasma viscosities at a shear rate of 150 s^{-1} at 37 °C at both native and standard Hct (40%). The animals were then sacrificed under anesthesia.

RBC deformability measurements

RBC deformability (i.e., the ability of the entire cell to adopt a new configuration when subjected to applied mechanical forces) was determined at various fluid shear stresses by laser diffraction analysis using an ektacytometer (LORCA, RR Mechatronics; Hoorn, The Netherlands). The system has been described elsewhere in detail (Hardeman et al., 1994). Briefly, a low hematocrit (Hct) suspension of RBC in an isotonic viscous medium (4% polyvinylpyrrolidone 360 solution; MW 360 kD, Sigma P 5288, ST. LOUIS, MI) was sheared in a Couette system composed of a glass cup and a precisely fitting bob, with a gap of 0.3 mm between the cylinders. A laser beam was directed through the sheared sample, and the diffraction pattern produced by the deformed cells was analyzed by a microcomputer. On the basis of the geometry of the elliptical diffraction pattern, an elongation index (EI) was calculated for shear rates between 0.3 and 30 Pa as: $EI=(L-W)/(L+W)$, where L and W are the length and width of the diffraction pattern, respectively. An increased EI at a given shear stress indicates greater cell deformation and hence greater RBC deformability. All measurements were carried out at 37 °C.

RBC aggregation measurements

RBC aggregation was also determined by LORCA as described elsewhere (Hardeman et al., 2001). The measurement is based on the detection of laser backscattering from the sheared (disaggregated), then unsheared (aggregating) blood, performed in a computer-assisted system at 37 °C. Backscattering data are evaluated by the computer and the aggregation index (AI),

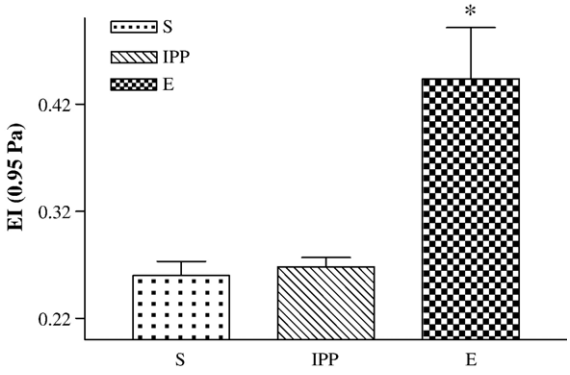

Fig. 1. RBC elongation index (EI) values of studied groups. EI was measured at a shear stress of 0.95 Pa. Values are expressed as means±SE. *: $p<0.001$ difference between the S and IPP groups.

Table 1
Plasma viscosity and aggregation parameters of experimental groups

	AI	t 1/2	γthr	PV	Tk
	(%)	(s)	(s^{-1})	(mPa s)	
S	53.66±2.11	3.37±0.42	119.16±6.75	1.26±0.01	1.17±0.017
IPP	61.96±2.24	2.16±0.26	105.00±4.18	1.32±0.04	1.06±0.060
E	56.03±2.08	2.91±0.39	110.00±6.70	1.26±0.01	1.09±0.041

AI, aggregation index; t 1/2, aggregation half time; γthr, threshold shear rate; PV, plasma viscosity measured at a shear rate of 150 s^{-1}; Tk, erythrocyte rigidity index.

aggregation half time (t 1/2) which shows the kinetics of aggregation and the threshold shear rate (γthr) which is a measure for the tendency to aggregate and aggregate stability are calculated on the basis that there is less light backscattered from aggregating red cells. The Hct of the samples used for aggregation measurements was adjusted to 40% and blood was fully oxygenated.

Determination of viscometric measurements

Whole blood viscosities (WBV) were determined with a Wells–Brookfield cone-plate rotational viscometer (model DV-II+Pro, with CPE40 spindle Brookfield engineering Labs, Middleboro, MA) at a shear rate of 150 s^{-1} at 37 °C at both native Hct and standard Hct (40%). PV was determined using the same viscometer at 150 s^{-1} at 37 °C. The viscometer is accurate for the shear rate used. The Hct of the blood samples was adjusted to 0.4 L/L by adding or removing a calculated amount of autologous plasma obtained by centrifugation at 1400 ×g for 6 min. The index of erythrocyte rigidity (Tk) was calculated at a shear rate of 150 s^{-1} according to the equation of Dintenfass, 1985:

$$Tk = (\eta r^{0.4} - 1)/(\eta r^{0.4} \cdot Hct),$$

where ηr was the relative blood viscosity (ηblood/ηplasma).

Statistical analysis

Results are expressed as means±SE. Statistical comparisons among groups were done by Kruskal–Wallis test and Mann–Whitney Utest after Bonferroni correction. p values <0.05 were considered statistically significant.

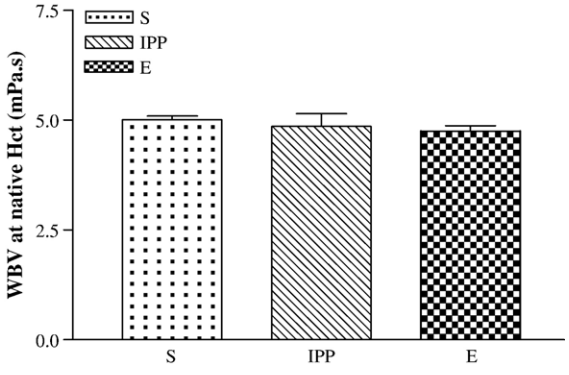

Fig. 2. Results of whole blood viscosity (WBV) at native hematocrit (Hct) in studied groups. WBV at native Hct was measured at a shear rate of 150 s^{-1}. Values are expressed as means±SE.

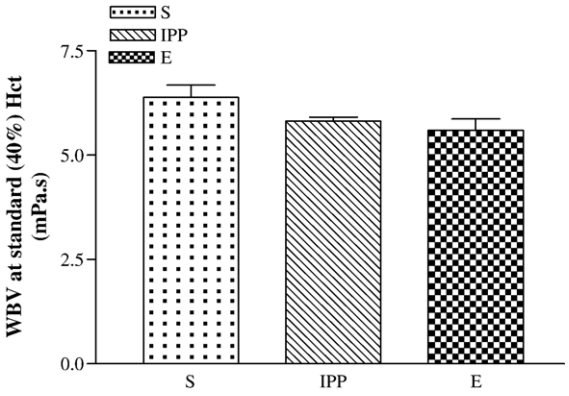

Fig. 3. Results of whole blood viscosity (WBV) at standard hematocrit (Hct, 40%) in studied groups. WBV at standard Hct was measured at a shear rate of 150 s^{-1}. Values are expressed as means±SE.

Results

RBC deformability (i.e., the elongation index EI) of RBCs in the S, IPP and E groups determined at a shear stress of 0.95 Pa are presented in Fig. 1. RBC deformability in the E group was found to be significantly increased compared with the S and IPP groups. Tk values which represent RBC rigidity were decreased in the E group compared to the S group, but this decrement was not found to be statistically significant. (Table 1, p>0.05). In order to eliminate the possible effects of penicillin G on RBC deformability, animals in the IPP group were treated by penicillin G intraperitoneally. No significant alteration in RBC deformability was observed in this group compared with the S group, suggesting that increased RBC deformability was related to the epileptic seizures in the E group.

The aggregation index (AI), aggregation half time (t 1/2), threshold shear rate (γthr) and PV of all experimental groups are presented in Table 1 indicating that there were no significant alterations in aggregation parameters and PV (at 150 s^{-1}) in any of the groups. Whole blood viscosities (WBV) were also measured at a shear rate of 150 s^{-1} at both native and standard Hct (40%) and presented in Figs. 2 and 3 respectively. Similarly, no statistically significant difference in WBVs was observed between groups at both native and standard Hct.

Discussion

Epilepsy is an episodic disorder of the nervous system arising from the excessively synchronous and sustained discharge of a group of nerve cells and one of the most common neurologic problems in the world (Meldrum and Bruton, 1992). In this study, hemorheological parameters such as RBC deformability, aggregation, PV and WBV at both native and standard Hct were examined in an experimental animal model of epilepsy. Epileptic seizures were induced by injecting penicillin G intracortically as stated earlier. This penicillin model has been one of the most important models for answering questions about epilepsy

(Fisher, 1989). Our study is the first report regarding the increased RBC deformability in rats during epileptic seizures. Elongation indices are given by LORCA a good, widely-used method to measure RBC deformability. But other methods (i.e., filtration, viscometry) to evaluate RBC deformability are still in use and different methods sometimes may give different results.

The results of this study demonstrated that RBC deformability significantly increases during seizures of penicillin epilepsy in rats (E group) compared to the S group. Although Tk values which represent RBC rigidity (i.e., 1/deformability) were decreased in the E group compared to the S group, this decrement was not statistically significant. The increment in RBC deformability in the E group can be explained as follows; there is broad agreement that epileptic seizures are associated with increases in regional CBF. It is generally accepted that increases in neuronal activity raises the cerebral metabolic rate of oxygen consumption, leading to an increase in CBF and cerebral blood volume (CBV) as the brain attempts to perfuse the active neurons with oxygenated hemoglobin (Shariff et al., 2006). Thus, it can be suggested that the increase of CBF may reflect neuronal activation. In general, the tissues in which the vasculature has sufficient regulatory ability can compensate their blood needs by an appropriate change of vascular geometry; besides, blood rheological parameters are also important factors for effective tissue perfusion (Baskurt and Meiselman, 2003). When considering the capillaries, RBC deformability should be the most important factor affecting the flow of blood, since erythrocytes must enter and transit by deforming in vessels smaller than their resting diameter (Secomb and Hsu, 1997; Parthasarathi and Lipowsky, 1999). The ability of the entire RBC to deform is of crucial importance for performing its function of oxygen delivery (Mohandas et al., 1983; Stuart and Nash, 1990). The inability to deform would make it difficult for the red cells to perform their function of oxygen delivery. A significant decrease in RBC deformability compromises blood flow through the microcirculation and curtails nutrient support (Mohandas et al., 1979; Chien, 1987). Thus, it seems to be logical that RBC deformability needs to be increased during epileptic seizures for the demands of neurons due to their enhanced activity. Although the underlying mechanisms in this observation are not clear, several factors could be involved in this regulation, such as products of neuronal metabolism (H+, adenosine, lactate), ions released upon neuronal activation, and/or neurotransmitters released close to the blood vessels (Lou et al., 1987; Brian et al., 1996; Gulbenkian et al., 2001). In addition, nitric oxide (NO) has been suggested to participate in the coupling between local perfusion and neural activity in various conditions (Dirnagl et al., 1993; Iadecola et al., 1994). Indeed, NO plays an important role in the regulation of cerebral circulation, both under basal conditions and during hypercapnia or local neuronal activation (Northington et al., 1992; Iadecola, 1992; Akgoren et al., 1994; Gotoh et al., 2001). NO participates also in the cerebrovascular response to focal seizures induced by bicuculline (Pereira de Vasconcelos et al., 1995) and to seizures induced by systemic kainate (KA) in the rat (Montecot et al., 1998; Sumanont et al., 2006). It is known that NO within a certain range yields to an increment in RBC deformability. NO synthesized in endothelial cells not only diffuses to the adjacent smooth muscle cells but also to the vascular lumen. In addition to its effects on leukocytes and platelets, NO interacts with RBCs and plays a very important role in preserving or enhancing RBC deformability (Korbut and Gryglewski, 1996; Starzyk et al., 1999; Bor-Kucukatay et al., 2000). Although NO levels were not measured in the current study, it can be speculated that, NO which is well-known to increase during epileptic seizures may be responsible for the enhancement in RBC deformability. However, it was also reported that NO at higher concentrations may also have deleterious effects on blood rheology (Korbut and Gryglewski, 1993; Brun, 2002; Bor-Kucukatay et al., 2003). The effects of NO on RBC deformability seem to be firmly concentration dependent.

In the present study, we did not find any significant differences in WBVs at both native and standard Hct in the experimental groups compared to the control. The resistance of blood to flow is known as blood viscosity. High blood viscosity slows down the blood flow. Normal erythrocytes are significantly deformable and due to the deformability of the erythrocytes, blood viscosity is lower than the viscosity of a fluid containing non-deformable particles of the same size. Blood viscosity is determined by Hct, PV, RBC aggregation and deformability. When erythrocytes become less deformable the viscosity of blood increases (Chien, 1975; Chien, 1987). Although we found highly significant increment in RBC deformability in the epileptic group compared with the S group, there were no significant changes in WBV in this group. In the light of the stated knowledge above, these parameters of blood should have decreased in harmony with increased RBC deformability but we found only a non-significant WBV decrement especially in epileptic rats and this observation remained to be clarified. Additionally, PV was also found to be unaltered.

Erythrocyte aggregation is the reversible adhesion of adjacent RBCs. The physiological importance of erythrocyte aggregation in circulation is its tendency to increase the blood viscosity in low shear flow and to disturb the passage in capillary circulation through the formation of sludge (Chien, 1975). In the present study, aggregation parameters such as the aggregation index (AI), aggregation half time (t 1/2) which shows the kinetics of aggregation and the threshold shear rate (γthr) which is a measure for the tendency to aggregate and aggregate stability were measured and no significant change was observed between the experimental groups.

In summary, we have observed that among the measured rheological parameters only RBC deformability is significantly increased during epileptic seizures. This observation could be related to an increase in NO production which has been postulated as a mediator of coupling between blood flow and neuronal activity in epileptic seizures. Further examination will be needed to explain this topic.

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